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<input type="checkbox"/>	L2	nerve growth factor-activated-protein kinase	0
<input type="checkbox"/>	L1	(NGF-activated protein kinase)	0

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☐ 1. Document ID: US 20020160933 A1

Using default format because multiple data bases are involved.

L3: Entry 1 of 3

File: PGPB

Oct 31, 2002

PGPUB-DOCUMENT-NUMBER: 20020160933

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020160933 A1

TITLE: Methods and compositions for producing a neurosalutary effect in a subject

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Benowitz, Larry I.	Newton Centre	MA	US	

US-CL-CURRENT: 514/1

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	RMIC	Draw Des
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☐ 2. Document ID: US 20020160933 A1

L3: Entry 2 of 3

File: DWPI

Oct 31, 2002

DERWENT-ACC-NO: 2003-328371

DERWENT-WEEK: 200331

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TITLE: Producing neurosalutary effect, and treating neurological disorder, in a subject, by administering a therapeutically effective amount of a compound that modulates the activity of N-kinase, to the subject

INVENTOR: BENOWITZ, L I

PRIORITY-DATA: 2001US-0949200 (September 7, 2001), 2000US-0656915 (September 7, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<u>US 20020160933 A1</u>	October 31, 2002		020	A61K031/00

INT-CL (IPC): A61 K 31/00

ABSTRACTED-PUB-NO: US20020160933A

BASIC-ABSTRACT:

NOVELTY - Producing (M1) a neurosalutary effect in a subject, and treating a subject

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suffering from neurological disorder, involves administering a therapeutically effective amount of a compound (I) that modulates the activity of N-kinase, to the subject.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

- (1) identifying (M2) a compound capable of producing a neurosalutary effect in a subject, by contacting N-kinase or its biologically active fragment, with a test compound and determining the ability of the test compound to modulate the activity of N-kinase;
- (2) a compound capable of producing a neurosalutary effect in a subject identified by the above method;
- (3) an isolated N-kinase polypeptide (II) of the type that:
 - (a) is present in neonatal brain tissue
 - (b) is inhibited in the presence of 6-thioguanine
 - (c) is activated in the presence of Mn²⁺ but not by Mg²⁺ or Ca²⁺
 - (d) has a molecular weight of 49 kDa, and
 - (e) is eluted from a Cibacron Blue column at a NaCl concentration of 1.5-1.75 M;
- (4) an antibody which is specifically reactive with an epitope of (II);
- (5) a fragment of (II) comprising at least 15 contiguous amino acids, and capable of eliciting an immune response; and
- (6) an isolated nucleic acid molecule (III) encoding a polypeptide comprising a sequence of 272 amino acids fully defined in the specification.

ACTIVITY - Anticonvulsant; Cerebroprotective; Neuroprotective; Nootropic.

No supporting biological data is given.

MECHANISM OF ACTION - Modulator of N-kinase activity (claimed); Promotes neuronal survival, axonal outgrowth and neuronal regeneration; Intracellular mediator of axonal outgrowth.

No supporting biological data is given.

USE - M1 is useful for producing a neurosalutary effect, and thus for treating a subject e.g. mammal, preferably human, suffering from neurological disorder such as spinal cord injury (including monoplegia, diplegia, paraplegia, hemiplegia and quadriplegia), epilepsy, stroke and Alzheimer's disease. The treatment method further involves making a first assessment of a nervous system function prior to administering (I) and making a second assessment of a nervous system function after administering (I) to the subject. The nervous system function is a sensory function, cholinergic innervation or vestibulomotor function (claimed).

(II) is useful as bait protein in a two- or three-hybrid assay, to identify other proteins, which bind to or interact with N-kinase.

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KNOC	Draw Des
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3. Document ID: JP 2004523470 W, WO 200220056 A2, AU 200187118 A, EP 1315514 A2

L3: Entry 3 of 3

File: DWPI

Aug 5, 2004

h e b b g e e f e c h e f b e

DERWENT-ACC-NO: 2002-393816

DERWENT-WEEK: 200451

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TITLE: Producing a neurosalutary effect in a subject e.g., one suffering from neurological disorder such as stroke, to treat the subject, by administering a compound that modulates activity of N-kinase

INVENTOR: BENOWITZ, L I

PRIORITY-DATA: 2000US-0656915 (September 7, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<u>JP 2004523470 W</u>	August 5, 2004		077	A61K045/00
<u>WO 200220056 A2</u>	March 14, 2002	E	042	A61K045/00
<u>AU 200187118 A</u>	March 22, 2002		000	A61K045/00
<u>EP 1315514 A2</u>	June 4, 2003	E	000	A61K038/18

INT-CL (IPC): A61 K 9/10; A61 K 9/127; A61 K 38/18; A61 K 45/00; A61 P 9/10; A61 P 9/12; A61 P 25/00; A61 P 25/02; A61 P 25/08; A61 P 25/14; A61 P 25/16; A61 P 25/18; A61 P 25/24; A61 P 25/28; A61 P 43/00; C07 K 14/475; C07 K 16/40; C12 N 9/12; C12 N 15/09; C12 Q 1/48; G01 N 33/15; G01 N 33/50; G01 N 33/53; G01 N 33/566

ABSTRACTED-PUB-NO: WO 200220056A

BASIC-ABSTRACT:

NOVELTY - Producing (M1) a neurosalutary effect in a subject e.g., a subject suffering from a neurological disorder, to treat the subject suffering from the neurological disorder, involving administering to the subject a compound (I) that modulates the activity of N-kinase, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) an isolated N-kinase polypeptide (II) of the type that: is present in neonatal brain tissue; is inhibited in the presence of 6-thioguanine; is activated in the presence of Mn²⁺, but not by Mg²⁺ or Ca²⁺; has a molecular weight of approximately 49 kDa; and is eluted from a Cibacron Blue column at a sodium chloride concentration of 1.5-1.75 M;
- (2) an antibody (III) which is specifically reactive with an epitope of (II);
- (3) a fragment (IV) of (I), which comprises at least 15 contiguous amino acids, and is able to elicit an immune response;
- (4) an isolated nucleic acid molecule that encodes (II); and
- (5) a compound capable of producing a neurosalutary effect in a subject identified using (II).

ACTIVITY - Nootropic; neuroprotective; cerebroprotective; anticonvulsant; vulnerary; tranquilizer; antiparkinsonian; antimanic; antidepressant.

MECHANISM OF ACTION - N-kinase activity modulator; neuronal survival modulator; neuronal regeneration modulator; neuronal axonal outgrowth of central nervous system neurons e.g., retinal ganglion cells, modulator (all claimed).

No data given.

USE - (I) is useful for producing a neurosalutary effect in a subject e.g., a subject suffering from a neurological disorder, to treat the subject (preferably, humans)

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suffering from the neurological disorder. The neurosalutary effect is produced by modulating neuronal survival, modulating neuronal regeneration or modulating neuronal axonal outgrowth of central nervous system neurons e.g., retinal ganglion cells, in a subject suffering from a neurological disorder such as spinal cord injury characterized by monoplegia, diplegia, paraplegia, hemoplegia and quadriplegia, or suffering from epilepsy, stroke or Alzheimer's disease.

(II) is useful for identifying a compound capable of producing a neurosalutary effect in a subject, preferably a compound which inhibits or stimulates the activity of N-kinase, which involves contacting (II) or its biologically active fragment with a test compound and determining the ability of the test compound to modulate the activity of N-kinase, thereby identifying a compound capable of producing a neurosalutary effect in a subject. The ability of the test compound to modulate the activity of N-kinase is determined by assessing the ability of the test compound to modulate N-kinase-dependant phosphorylation of a substrate. Optionally, (I) is identified using (II) by the following method which involves contacting (II) or its biologically active fragment, with a test compound, an N-kinase substrate (e.g., histone H1 protein), radioactive ATP (preferably gamma -32P), and Mn2+; and determining the ability of the test compound to modulate N-kinase dependent phosphorylation of the substrate, thereby identifying a compound capable of producing a neurosalutary effect in a subject. (II) used in the methods described above is preferably a recombinantly produced human N-kinase. Optionally, (II) is bovine N-kinase purified from a bovine source. The methods further involve determining the ability of the test compound to modulate axonal outgrowth of central nervous system neuron (all claimed).

(M1) is useful for treating a neurological disorder such as dementia's related to Alzheimer's disease, Parkinson's disease, senile dementia, Huntington's disease, Creutzfeldt-Jakob disease, Korsakoff's psychosis, mania, anxiety disorders, obsessive-compulsive disorder, anxiety, bipolar affective disorder. The methods are useful for preventing or treating neurological deficits in embryos or fetuses in utero, in premature infants, or in children with need of such treatment, including those with neurological birth defects. (I) is also useful for modulating activity of N-kinase, in vitro to modulate axonal outgrowth in vitro.

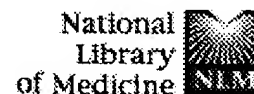
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N-kinase	3

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☐ **3:** Vaskovsky A, Lupowitz Z, Erlich S, Pinkas-Kramarski R. Related Articles, Li

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Neuroscience. 1999 Jan;88(2):405-24.
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Proc Natl Acad Sci U S A. 1997 Sep 2;94(18):9909-14.
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

J Neurosci Res. 1995 Jan 1;40(1):108-16.

PMID: 7714918 [PubMed - indexed for MEDLINE]

 10: [Loeb DM, Tsao H, Cobb MH, Greene LA.](#)[Related Articles, Li](#) NGF and other growth factors induce an association between ERK1 and the NGF receptor, gp140prototr.



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J Biol Chem. 1992 Oct 25;267(30):21663-70.

PMID: 1400478 [PubMed - indexed for MEDLINE]

 12: [Volonte C, Greene LA.](#)[Related Articles, Li](#) 6-Methylmercaptopurine riboside is a potent and selective inhibitor of nerve growth factor-activated protein kinase N.



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 14: [Rowland EA, Muller TH, Goldstein M, Greene LA.](#)[Related Articles, Li](#) Cell-free detection and characterization of a novel nerve growth factor-activated protein kinase in PC12 cells.

J Biol Chem. 1987 Jun 5;262(16):7504-13.

PMID: 3584124 [PubMed - indexed for MEDLINE]

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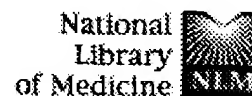
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Cell-free detection and characterization of a novel nerve growth factor-activated protein kinase in PC12 cells.

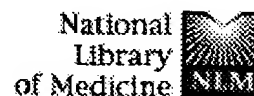
Rowland EA, Muller TH, Goldstein M, Greene LA.

We have developed a cell-free assay to detect and characterize nerve growth factor (NGF)-activated protein kinase activity. Cultured PC12 cells were briefly exposed to NGF, and extracts of these were assayed for phosphorylating activity using exogenously added tyrosine hydroxylase as substrate. Tyrosine hydroxylase was employed since it is an endogenous substrate of NGF-regulated kinase activity and is activated by phosphorylation. In the cell-free assay, extracts prepared from NGF-treated cells yielded a 2-3-fold greater incorporation of phosphate into tyrosine hydroxylase as compared with extracts of control, NGF untreated cells. Activation did not occur, however, if NGF was added directly to cell extracts. The NGF-stimulated phosphorylating activity appeared to be due to regulation of a protein kinase rather than of a phosphoprotein phosphatase. Characterization of the kinase (designated as kinase N) showed that it is soluble, is detectably activated within 1-3 min after cells are exposed to NGF and maximally activated by 10 min, is half-maximally activated with 0.5 nM NGF and maximally activated with 1 nM NGF, is detectable in the presence of either Mg^{2+} or Mn^{2+} but does not require Ca^{2+} , does not require nonmacromolecular cofactors, can use histone H1 as a substrate, and exhibits a 2-fold increase in apparent V_{max} in response to NGF but does not undergo a significant change in apparent K_m for either ATP or GTP. A number of characteristics of kinase N were assessed including susceptibility to inhibitors, substrate specificity, cofactor requirements, ATP dependence, and lack of down-regulation by prolonged exposure to a phorbol ester. These studies indicated that it lacks tyrosine kinase activity and is distinct from a variety of well-characterized protein kinases including cAMP-dependent protein kinase, protein kinase C (Ca^{2+} /phospholipid dependent enzyme), Ca^{2+} /calmodulin-dependent kinase, and casein kinase II. Preliminary purification data show that the kinase has a basic pI and that it has an apparent M_r of 22,000-25,000. The only amino acid in tyrosine hydroxylase found to be phosphorylated by the semipurified kinase is serine.

PMID: 3584124 [PubMed - indexed for MEDLINE]

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Nerve growth factor-activated protein kinase N. Characterization and rapid near homogeneity purification by nucleotide affinity-exchange chromatography.

Volonte C, Greene LA.

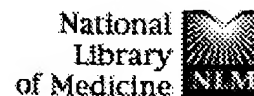
Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, New York 10032.

Protein kinase N (PKN) is a protein kinase rapidly activated by nerve growth factor (NGF) and other agents in PC12 pheochromocytoma and additional cell types. PKN is selectively inhibited by purine analogs, and this property has served both as a diagnostic for PKN activity and to establish its apparent involvement in certain pathways of the NGF mechanism of action. The present work has focused on further characterization, identification, and purification of NGF-activated PKN. We show here that PKN can be substantially enriched by elution from ion exchange resins with ATP. We exploited this novel technique (nucleotide affinity exchange chromatography) to devise two alternative isolation schemes for PKN. One utilizes sequential chromatographic steps and provides preparation that is apparently 60% homogeneous for PKN and represents a total enrichment of approximately 10,000-fold. The other is a single column procedure and includes prewashes with NAD. This method yields material that is about 5-10% homogeneous for PKN, requires about 1 h, and can be applied to multiple samples in parallel. The ATP elution technique furthermore distinguishes NGF-regulated from basal PKN activity and thereby suggests the presence of distinct PKN isoforms. The applications of sucrose gradient centrifugation, gel filtration chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/silver staining, affinity labeling with 8-azido-ATP/SDS-PAGE, autophosphorylation (after SDS-PAGE, blotting and renaturation) all indicate PKN has an apparent molecular mass of 45-47 kDa and is mainly monomeric in solution. These and additional properties appear to distinguish PKN from many previously described protein kinases.

PMID: 1400478 [PubMed - indexed for MEDLINE]

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Nerve growth factor-activated protein kinase N modulates the cAMP-dependent protein kinase.

Volonte C, Greene LA.

Department of Pathology, College of Physicians and Surgeons of Columbia University, New York, New York.

Protein kinase N (PKN) is a serine/threonine protein kinase rapidly activated by nerve growth factor (NGF) and other agents in various cell lines. The possible involvement of PKN in the multiple pathways of the NGF mechanism of action was previously established through the use of purine analogs, some of which are apparently specific inhibitors of this kinase. Since a PKN-like activity is modulated in several cell lines by cAMP analogs and this activation requires the activity of cAMP-dependent protein kinase, the aim of the present work is to investigate possible interactions between PKN and C-PKA. Pre-incubation of the two kinases in the presence of ATP leads to potentiated phosphorylation of histone H1, Kemptide (a substrate for C-PKA, but not for PKN), and several additional substrates. This augmented phosphorylating activity is insensitive to thioguanine (an inhibitor for PKN, but not for C-PKA) and is suppressed both by the Walsh inhibitor and by the regulatory subunit of PKA. PKN-pretreated C-PKA shows a significant decrease in K_m for Kemptide and a substantial increase in V_{max} . C-PKA and PKN are widely expressed enzymes and the possibility of PKN-dependent modulation of PKA in intact cells would therefore have biological implications for signal transduction mechanisms.

PMID: 7714918 [PubMed - indexed for MEDLINE]

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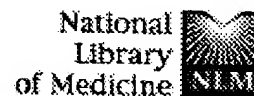
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A signaling organelle containing the nerve growth factor-activate receptor tyrosine kinase, TrkA.

Grimes ML, Beattie E, Mobley WC.

Department of Biochemistry, Massey University, Palmerston North, New Zealand. M.L.Grimes@Massey.ac.NZ

The topology of signal transduction is particularly important for neurons. Neurotrophic factors such as nerve growth factor (NGF) interact with receptor distal axons and a signal is transduced by retrograde transport to the cell body ensure survival of the neuron. We have discovered an organelle that may acco for the retrograde transport of the neurotrophin signal. This organelle is derive from endocytosis of the receptor tyrosine kinase for NGF, TrkA. In vitro reactions containing semi-intact PC12 cells and ATP were used to enhance recovery of a novel organelle: small vesicles containing internalized NGF bou to activated TrkA. These vesicles were distinct from clathrin coated vesicles, uncoated primary endocytic vesicles, and synaptic vesicles, and resembled transport vesicles in their sedimentation velocity. They contained 10% of the t bound NGF and almost one-third of the total tyrosine phosphorylated TrkA. These small vesicles are compelling candidates for the organelles through whi the neurotrophin signal is conveyed down the axon.

PMID: 9275225 [PubMed - indexed for MEDLINE]

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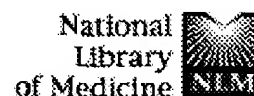
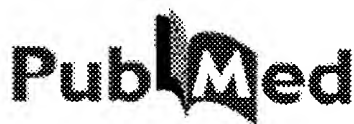
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


















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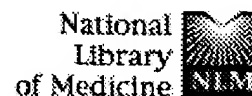
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Nerve growth factor (NGF) responses by non-neuronal cells: detection by assay of a novel NGF-activated protein kinase.

Volonte C, Greene LA.

Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, New York 10032.

Past work described the partial purification and characterization of a novel serine protein kinase activity designated protein kinase N (PKN) that is activated by nerve growth factor (NGF) in cultured PC12 cells [Rowland et al. (1987) J. Biol. Chem. 262; 7504-7513]. We have now devised a rapid, sensitive technique for partially purifying and assaying PKN activity in cell extracts. This methodology was applied to the IARC-EW-1 osteosarcoma and several additional non-neuronal cell lines that possess NGF receptors but that lack both morphological and a variety of additional biochemical responses to NGF. In each case, NGF significantly elevated PKN activity. The assay also revealed activation of PKN activity in IARC-EW-1 cells by additional agents, including epidermal growth factor, fibroblast growth factor, phorbol ester, and a cAMP analog. Also tested were an NGF-receptor-deficient PC12 cell variant and sublines thereof into which human NGF receptors had been introduced [Hempstead et al. (1989) Science 253:373-375]. Acquisition of the NGF receptors resulted in NGF-activatable PKN activity. These findings indicate that detection of PKN activity may serve as a sensitive means to test NGF responsiveness in cells lacking macroscopic responses to the factor and that non-neuronal cells may be useful for studying primary signaling events in the NGF mechanism of action.

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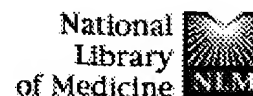
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
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
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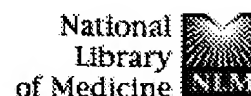
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jun-NH2-terminal kinase activation mediated by UV-induced DNA lesions in melanoma and fibroblast cells.

Adler V, Fuchs SY, Kim J, Kraft A, King MP, Pelling J, Ronai Z.

Molecular Carcinogenesis Program, American Health Foundation, Valhalla, N York 10595, USA.

jun-NH2-terminal kinase (JNK) belongs to a family of protein kinases that phosphorylates c-Jun, ATF2, and Elk1 in response to various forms of stress including UV irradiation and heat shock. Although in previous studies we have demonstrated the importance of membrane components for JNK activation by irradiation, here we have elucidated the role of DNA damage in this response. We show that in vitro-irradiated or sonicated DNA that is added to proteins prepared from UV-treated cells can further induce JNK activation in a dose-dependent manner. When compared with UV-B (300 nm), UV-C (254 nm), which is better absorbed by the DNA, is significantly more potent in activating JNK. Furthermore, when wavelengths lower than 300 nm were filtered out, UV-B was no longer able to activate JNK. With the aid of melanoma and fibroblast cells, which exhibit different resistances to irradiation and require different UV doses to generate the same number of DNA lesions, we demonstrate that above a threshold level of 0.45 lesions and up to 0.75 lesions per 1875 bp, the degree of JNK activation correlates with the amount of lesions induced by UV-C irradiation. Finally, to explore the role of nuclear and mitochondrial DNA (mtDNA) in mediating JNK activation after UV irradiation, we have used cells that lack mtDNA. Although the lack of mtDNA did not impair the ability of UV to activate JNK, when enucleated, these cells had lost the ability to activate JNK in response to UV irradiation. Overall, our results suggest that DNA damage in the nuclear compartment is an essential component that acts in concert with membrane-anchored proteins to mediate c-Jun phosphorylation by JNK.

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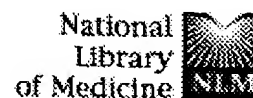
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UV irradiation and heat shock mediate JNK activation via altern: pathways.

Adler V, Schaffer A, Kim J, Dolan L, Ronai Z.

Molecular Carcinogenesis Program, American Health Foundation, Valhalla, N York 10595, USA.

To elucidate cellular pathways involved in Jun-NH2-terminal kinase (JNK) activation by different forms of stress, we have compared the effects of UV irradiation, heat shock, and H₂O₂. Using mouse fibroblast cells (3T3-4A) we show that while H₂O₂ is ineffective, UV and heat shock (HS) are potent induc of JNK. The cellular pathways that mediate JNK activation after HS or UV exposure are distinctly different as can be concluded from the following observations: (i) H₂O₂ is a potent inhibitor of HS-induced but not of UV-indu JNK activation; (ii) Triton X-100-treated cells abolish the ability of UV, but not HS, to activate JNK; (iii) the free radical scavenger N-acetylcysteine inhibits U but not HS-mediated JNK activation; (iv) N-acetylcysteine inhibition is blocke by H₂O₂ in a dose-dependent manner; (v) a Cockayne syndrome-derived cell exhibits JNK activation upon UV exposure, but not upon HS treatment. The significance of Jun phosphorylation by JNK after treatment with UV, HS, or H₂O₂ was evaluated by measuring Jun phosphorylation in vivo and also its binding activity in gel shifts. HS and UV, which are potent inducers of JNK, increased the level of c-Jun phosphorylation when this was measured by [32P] orthophosphate labeling of 3T3-4A cultures. H₂O₂ had no such effect. Althou, H₂O₂ failed to activate JNK in vitro and to phosphorylate c-Jun in vivo, all th forms of stress were found to be potent inducers of binding to the AP1 target sequence. Overall, our data indicate that both membrane-associated compon and oxidative damage are involved in JNK activation by UV irradiation, where HS-mediated JNK activation, which appears to be mitochondrial-related, utiliz cellular sensors.

PMID: 7592807 [PubMed - indexed for MEDLINE]

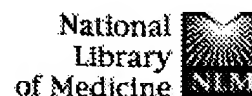
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J Biol Chem. 1994 Apr 15;269(15):11186-91.

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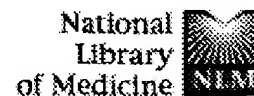
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A peptide encoding the c-Jun delta domain inhibits the activity of c-jun amino-terminal protein kinase.

Adler V, Unlap T, Kraft AS.

Division of Hematology/Oncology, University of Alabama, Birmingham 3529

Evidence suggests that the c-Jun protooncogene delta (delta) domain (amino acids 31-60) helps regulate the transcriptional activating capacity of c-Jun by modulating the amino-terminal phosphorylation of this protein. By using a peptide encoding the delta domain and purified amino-terminal c-Jun protein kinase, we demonstrate that the delta domain peptide inhibits phosphorylation of the amino terminus of both c-Jun and the related protein JunD. The delta domain peptide inhibited the activation of the c-Jun amino-terminal protein kinase by phorbol esters in permeabilized U937 leukemic cells. Mutation of c-Jun followed by transfection into U937 leukemic cells demonstrated that partial deletions of the delta domain are sufficient to block phosphorylation of the amino terminus of c-Jun. In vitro deletion of the amino-terminal (amino acids 31-44) half of the delta domain inhibited the phosphorylation of c-Jun. However, deletion of the carboxyl-terminal (amino acids 45-60) half only partially inhibited c-Jun phosphorylation. Therefore, these results indicate that the delta domain sequence is an important regulator of c-Jun amino-terminal phosphorylation.

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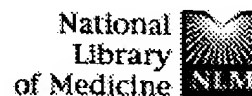
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J Biol Chem. 1992 Aug 25;267(24):17001-5.
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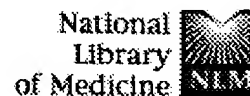
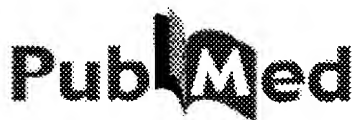
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Affinity-purified c-Jun amino-terminal protein kinase requires serine/threonine phosphorylation for activity.

Adler V, Polotskaya A, Wagner F, Kraft AS.

Division of Hematology/Oncology, University of Alabama, Birmingham 3529

The addition of phorbol esters to U937 leukemic cells stimulates the phosphorylation of c-Jun on serines 63 and 73. To isolate the protein kinase which stimulates this phosphorylation, we have used heparin-Sepharose chromatography followed by affinity chromatography over glutathione-Sepharose beads bound with a fusion protein of glutathione S-transferase and amino acid: 89 of c-Jun (GST-c-Jun). Using this procedure we purify a 67-kDa protein which is capable of phosphorylating GST-c-Jun as well as the complete c-Jun protein. By making mutations in serines 63 and 73 and then creating a fusion protein with GST (GST-c-Jun mut), we demonstrate that this protein kinase specifically phosphorylates these sites in the c-Jun amino terminus. Treatment of purified c-Jun amino-terminal protein kinase (cJAT-PK) with phosphatase 2A inhibits its ability to phosphorylate GST-c-Jun. This inactivated enzyme can be reactivated by phosphorylation with protein kinase C (PKC), although PKC is not capable of phosphorylating the GST-c-Jun substrate. Because v-Jun cannot be phosphorylated in vivo, we compared the ability of cJAT-PK to bind to GST-v-Jun or GST-c-Jun mut. The cJAT-PK bound 50-fold better to GST-c-Jun mut than to GST-v-Jun suggesting that the delta domain which is missing in v-Jun plays a role in binding the cJAT-PK. These results suggest that there is a protein kinase cascade mediated by protein phosphatases and PKC which regulates c-Jun phosphorylation.

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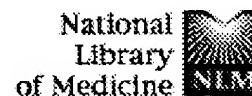
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





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



















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



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Human leukemic models of myelomonocytic development: a review of the F 60 and U937 cell lines.

J Leukoc Biol. 1985 Apr;37(4):407-22.

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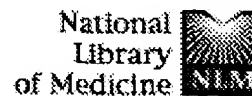
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1: Zhou TH, Ling K, Guo J, Zhou H, Wu YL, Jing Q, Ma L, Pei G.

Related Articles, Li



Identification of a human brain-specific isoform of mammalian STE20-like kinase 3 that is regulated by cAMP-dependent protein kinase.

J Biol Chem. 2000 Jan 28;275(4):2513-9.

PMID: 10644707 [PubMed - indexed for MEDLINE]

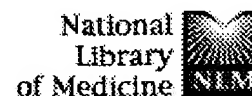
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Identification of a human brain-specific isoform of mammalian STE20-like kinase 3 that is regulated by cAMP-dependent protein kinase.

Zhou TH, Ling K, Guo J, Zhou H, Wu YL, Jing Q, Ma L, Pei G.

Shanghai Institute of Cell Biology, Chinese Academy of Sciences, 320 Yue Y Road, Shanghai 200031, People's Republic of China.

A novel isoform of mammalian STE20-like kinase 3 (MST3) with a different coding region from MST3, termed MST3b, was identified by searching through expressed sequence tag data base and obtained by rapid amplification of cDNA 5'-ends. MST3b was assigned to the long arm of human chromosome 13, D13S159-D13S280, by use of the National Center for Biotechnology Information sequence-tagged sites data base. Reverse transcription-polymerase chain reaction and Northern blot analysis with a probe derived from 5' distinct sequence of MST3b revealed that the expression of MST3b mRNA is restricted to the brain in contrast to ubiquitous distribution of MST3 transcript. Western analysis confirmed the brain-specific expression of MST3b protein. In situ hybridization of rat brain sections with a MST3b-specific probe indicated that MST3b is widely expressed in different brain regions, with especially high expression in hippocampus and cerebral cortex. When expressed in human embryonic kidney 293 (HEK293) cells, MST3b effectively phosphorylated myelin basic protein, well as undergoing autophosphorylation. Interestingly, expression of MST3, but not MST3b, in HEK293 cells was able to activate the endogenous p42/44 mitogen-activated protein kinase (MAPK) up to 4-fold, whereas neither isoform activated p38 MAPK under the same conditions. Further experiments demonstrated that MST3b, but not MST3, was effectively phosphorylated by activation of cyclic AMP-dependent protein kinase (PKA) in both in vivo and vitro assays. The mutation of Thr-18 into Ala in MST3b (T18A), a putative PK phosphorylation site that is absent in MST3, abolished its phosphorylation by PKA. Consequently, expression of the T18A mutant in HEK293 cells led to partial activation of p42/44 MAPK, indicating that MST3b is under the regulation of PKA. Taken together, our data provide evidence that the two isoforms of STE20-like kinase 3 are differentially distributed and regulated.

PMID: 10644707 [PubMed - indexed for MEDLINE]

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AN 2004:345810 BIOSIS

DN PREV200400346959

TI Nerve growth factor promotes the survival of sympathetic neurons through
the cooperative function of the protein kinase C and phosphatidylinositol
3-kinase pathways.

AU Pierchala, Brian A. [Reprint Author]; Ahrens, Rebecca C.; Paden, Andrew
J.; Johnson, Eugene M. Jr

CS Sch MedDept Mol Biol and Pharmacol, Washington Univ, 4566 Scott Ave, Box
8103, St Louis, MO, 63110, USA
btp@msnotes.wustl.edu

S0 Journal of Biological Chemistry, (July 2 2004) Vol. 279, No. 27, pp.
27986-27993. print.

CODEN: JBCHA3. ISSN: 0021-9258.

DT Article

LA English

ED Entered STN: 18 Aug 2004

Last Updated on STN: 18 Aug 2004

L4 ANSWER 2 OF 23 USPATFULL on STN

AN 2002:27124 USPATFULL

TI Novel methods of diagnosis of angiogenesis, compositions, and methods of
screening for angiogenesis modulators

IN Murray, Richard, Cupertino, CA, UNITED STATES

Watson, Susan, El Cerrito, CA, UNITED STATES

Weiss, Stephen J., Ann Arbor, MI, UNITED STATES

Glynne, Richard, Palo Alto, CA, UNITED STATES

Hevezi, Peter, San Francisco, CA, UNITED STATES

PI US 2002015970 A1 20020207
AI US 2000-738877 A1 20001215 (9)
RLI Continuation-in-part of Ser. No. US 2000-637977, filed on 11 Aug 2000,
PENDING
PRAI WO 2000-US22061 20000811
US 1999-148425P 19990811 (60)
DT Utility
FS APPLICATION
LN.CNT 3077
INCL INCLM: 435/007.230
INCLS: 435/006.000; 424/001.490; 424/178.100
NCL NCLM: 435/007.230
NCLS: 435/006.000; 424/001.490; 424/178.100
IC [7]
ICM: A61K051-00
ICS: C12Q001-68; G01N033-574; A61K039-395
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AN 1995:209893 BIOSIS
DN PREV199598224193
TI ***Nerve*** ***growth*** ***factor*** - ***activated***
protein ***kinase*** N modulates the cAMP-dependent protein
kinase.
AU Volonte, C. [Reprint author]; Greene, L. A.
CS Inst. Neurobiol., CNR, Viale Marx 15, 00137 Rome, Italy
SO Journal of Neuroscience Research, (1995) Vol. 40, No. 1, pp. 108-116.
CODEN: JNREDK. ISSN: 0360-4012.
DT Article
LA English
ED Entered STN: 23 May 1995
Last Updated on STN: 9 Jun 1995

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STN DUPLICATE 3
AN 1995:224826 BIOSIS
DN PREV199598239126
TI Stimulation of vgf gene expression by NGF is mediated through multiple
signal transduction pathways involving protein phosphorylation.
AU Salton, Stephen R. J. [Reprint author]; Volonte, Cinzia; D'Arcangelo,
Gabriella
CS Fishberg Res. Cent. Neurobiol., Mt. Sinai Sch. Med., Box 1065, One Gustave
Levy Place, New York, NY 10029-6574, USA
SO FEBS Letters, (1995) Vol. 360, No. 2, pp. 106-110.
CODEN: FEBLAL. ISSN: 0014-5793.
DT Article
LA English
ED Entered STN: 31 May 1995
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STN DUPLICATE 4
AN 1993:412748 BIOSIS
DN PREV199396078473
TI A purine analog-sensitive protein kinase activity associates with Trk
nerve growth factor receptors.
AU Volonte, Cinzia [Reprint author]; Loeb, David M.; Greene, Lloyd A.
CS Inst. Neurobiol., CNR, Viale Marx, 15, 00156 Rome, Italy
SO Journal of Neurochemistry, (1993) Vol. 61, No. 2, pp. 664-672.
CODEN: JONRA9. ISSN: 0022-3042.
DT Article
LA English
ED Entered STN: 8 Sep 1993
Last Updated on STN: 3 Jan 1995

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AN 1993:248429 CAPLUS
DN 118:248429
TI Association of a purine-analog-sensitive protein kinase activity with p75
nerve growth factor receptors
AU Volonte, Cinzia; Ross, Alonzo H.; Greene, Lloyd A.
CS Coll. Physicians Surg., Columbia Univ., New York, NY, 10032, USA
SO Molecular Biology of the Cell (1993), 4(1), 71-8
CODEN: MBCEEV; ISSN: 1059-1524
DT Journal

LA English

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AN 1993:7506 BIOSIS

DN PREV199395007506

TI ***Nerve*** ***growth*** ***factor*** - ***activated***
protein ***kinase*** N: Characterization and rapid near
homogeneity purification by nucleotide affinity exchange chromatography.

AU Volonte, Cinzia [Reprint author]; Greene, Lloyd A.

CS Institute Neurobiology, CNR, Viale Marx 15, 00156 Rome, Italy

SO Journal of Biological Chemistry, (1992) Vol. 267, No. 30, pp. 21663-21670.
CODEN: JBCHA3. ISSN: 0021-9258.

DT Article

LA English

ED Entered STN: 10 Dec 1992
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AN 1992:145774 BIOSIS

DN PREV199293079999; BA93:79999

TI 6 METHYLMERCAPTOPYRINE RIBOSIDE IS A POTENT AND SELECTIVE INHIBITOR OF
NERVE ***GROWTH*** ***FACTOR*** - ***ACTIVATED***
PROTEIN ***KINASE*** N.

AU VOLONTE C [Reprint author]; GREENE L A

CS DEP PATHOLOGY, COLLEGE PHYSICIANS SURGEONS COLUMBIA UNIVERSITY, 630 WEST
168TH STREET, NEW YORK, NY 10032, USA

SO Journal of Neurochemistry, (1992) Vol. 58, No. 2, pp. 700-708.
CODEN: JONRA9. ISSN: 0022-3042.

DT Article

FS BA

LA ENGLISH

ED Entered STN: 12 Mar 1992
Last Updated on STN: 13 Mar 1992

L4 ANSWER 9 OF 23 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN DUPLICATE 7

AN 91102585 EMBASE

DN 1991102585

TI Cick sympathetic neurons in culture respond differentially to nerve growth
factor and conditioned medium from activated splenic lymphocytes.

AU Luo J.-J.; Hasegawa S.

CS Center for Neurobiology, and Molecular Immunology, Chiba University Sch.
of Med., Inohana 1-8-1, Chiba 280, Japan

SO Neuroscience Research, (1991) 10/2 (137-148).
ISSN: 0168-0102 CODEN: NERADN

CY Ireland

DT Journal; Article

FS 021 Developmental Biology and Teratology
037 Drug Literature Index

LA English

SL English

L4 ANSWER 10 OF 23 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 8

AN 1990:379641 BIOSIS

DN PREV199090066322; BA90:66322

TI INDUCTION OF ORNITHINE DECARBOXYLASE BY NERVE GROWTH FACTOR IN PC12 CELLS
DISSECTION BY PURINE ANALOGUES.

AU VOLONTE C [Reprint author]; GREENE L A

CS DEP PATHOL, CENT NEUROBIOL BEHAVIOUR, COLL PHYSICIANS SURGEONS, COLUMBIA
UNIV, NEW YORK, NY 10032, USA

SO Journal of Biological Chemistry, (1990) Vol. 265, No. 19, pp. 11050-11055.
CODEN: JBCHA3. ISSN: 0021-9258.

DT Article

FS BA

LA ENGLISH

ED Entered STN: 21 Aug 1990
Last Updated on STN: 22 Aug 1990

L4 ANSWER 11 OF 23 CAPLUS COPYRIGHT 2004 ACS on STN

AN 1990:152533 CAPLUS

DN 112:152533

TI Nerve growth factor stimulates a protein kinase in PC-12 cells that
phosphorylates microtubule-associated protein-2

AU Miyasaka, Tadayo; Chao, Moses V.; Sherline, Peter; Saltiel, Alan R.
CS Lab. Mol. Oncol., Rockefeller Univ., New York, NY, 10021, USA
SO Journal of Biological Chemistry (1990), 265(8), 4730-5
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English

L4 ANSWER 12 OF 23 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on
STN
AN 1991:149500 BIOSIS
DN PREV199140069105; BR40:69105
TI MOLECULAR CHARACTERISTICS OF AN ***NGF*** - ***ACTIVATED***
PROTEIN ***KINASE*** PKN.
AU VOLONTE C [Reprint author]; GREENE L A
CS DEP PATHOL, COLUMBIA UNIV, NEW YORK, NY 10032, USA
SO Society for Neuroscience Abstracts, (1990) Vol. 16, No. 1, pp. 825.
Meeting Info.: 20TH ANNUAL MEETING OF THE SOCIETY FOR NEUROSCIENCE, ST.
LOUIS, MISSOURI, USA, OCTOBER 28-NOVEMBER 2, 1990. SOC NEUROSCI ABSTR.
ISSN: 0190-5295.
DT Conference; (Meeting)
FS BR
LA ENGLISH
ED Entered STN: 23 Mar 1991
Last Updated on STN: 22 May 1991

L4 ANSWER 13 OF 23 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 9
AN 1990:130545 BIOSIS
DN PREV199089069356; BA89:69356
TI MULTIPLE PATHWAYS OF N KINASE ACTIVATION IN PC12 CELLS.
AU ROWLAND-GAGNE E [Reprint author]; GREENE L A
CS DEPARTMENT PATHOLOGY, COLUMBIA UNIVERSITY, 630 WEST 168 STREET, NEW YORK,
NY 10032, USA
SO Journal of Neurochemistry, (1990) Vol. 54, No. 2, pp. 424-433.
CODEN: JONRA9. ISSN: 0022-3042.
DT Article
FS BA
LA ENGLISH
ED Entered STN: 13 Mar 1990
Last Updated on STN: 13 Mar 1990

L4 ANSWER 14 OF 23 CANCERLIT on STN DUPLICATE 10
AN 90132665 CANCERLIT
DN 90132665 PubMed ID: 2153751
TI Multiple pathways of N-kinase activation in PC12 cells.
AU Rowland-Gagne E; Greene L A
CS Department of Pharmacology, New York University School of Medicine.
NC GM 07238 (NIGMS)
NS16036 (NINDS)
SO JOURNAL OF NEUROCHEMISTRY, (1990 Feb) 54 (2) 423-33.
Journal code: 2985190R. ISSN: 0022-3042.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS MEDLINE; Priority Journals
OS MEDLINE 90132665
EM 199002
ED Entered STN: 19941107
Last Updated on STN: 19970509

L4 ANSWER 15 OF 23 CANCERLIT on STN DUPLICATE 11
AN 90248158 CANCERLIT
DN 90248158 PubMed ID: 2159763
TI Nerve growth factor (NGF) responses by non-neuronal cells: detection by
assay of a novel ***NGF*** - ***activated*** ***protein***
kinase.
AU Volonte C; Greene L A
CS Department of Pathology, College of Physicians and Surgeons, Columbia
University, New York, New York 10032.
NC NS16036 (NINDS)
SO GROWTH FACTORS, (1990) 2 (4) 321-31.
Journal code: 9000468. ISSN: 0897-7194.
CY Switzerland
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS MEDLINE; Priority Journals

OS MEDLINE 90248158
EM 199006
ED Entered STN: 19941107
Last Updated on STN: 19970509

L4 ANSWER 16 OF 23 CANCERLIT on STN
AN 90657021 CANCERLIT
DN 90657021
TI THE CHARACTERIZATION, PARTIAL PURIFICATION AND REGULATION OF AN
NGF - ***ACTIVATED*** ***PROTEIN*** ***KINASE*** IN PC12
CELLS.
AU Gagne E R
CS New York Univ., NY.
SO Diss Abstr Int [B], (1989) 49 (9) 3551.
ISSN: 0419-4217.
DT (THESIS)
LA English
FS Institute for Cell and Developmental Biology
EM 198912
ED Entered STN: 19941107
Last Updated on STN: 19970509

L4 ANSWER 17 OF 23 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 12
AN 1990:30235 BIOSIS
DN PREV199089017201; BA89:17201
TI DIFFERENTIAL INHIBITION OF NERVE GROWTH FACTOR RESPONSES BY PURINE
ANALOGUES CORRELATION WITH INHIBITION OF A ***NERVE*** ***GROWTH***
FACTOR - ***ACTIVATED*** ***PROTEIN*** ***KINASE*** .
AU VOLONTE C [Reprint author]; RUKENSTEIN A; LOEB D M; GREENE L A
CS DEP PATHOL, COLL PHYSICIANS SURG COLUMBIA UNIV, NEW YORK 10032, USA
SO Journal of Cell Biology, (1989) Vol. 109, No. 5, pp. 2395-2404.
CODEN: JCLBA3. ISSN: 0021-9525.
DT Article
FS BA
LA ENGLISH
ED Entered STN: 19 Dec 1989
Last Updated on STN: 20 Dec 1989

L4 ANSWER 18 OF 23 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
on STN
AN 89:568995 SCISEARCH
GA The Genuine Article (R) Number: AX799
TI DIFFERENTIAL INHIBITION OF NERVE GROWTH-FACTOR RESPONSES BY PURINE ANALOGS
- CORRELATION WITH INHIBITION OF A ***NERVE*** ***GROWTH*** -
FACTOR ***ACTIVATED*** ***PROTEIN*** - ***KINASE***
AU VOLONTE C (Reprint); RUKENSTEIN A; LOEB D M; GREENE L A
CS COLUMBIA UNIV COLL PHYS & SURG, DEPT PATHOL, NEW YORK, NY, 10032
(Reprint); COLUMBIA UNIV COLL PHYS & SURG, CTR NEUROBIOL & BEHAV, NEW
YORK, NY, 10032
CYA USA
SO JOURNAL OF CELL BIOLOGY, (1989) Vol. 109, No. 5, pp. 2395-2403.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 41

L4 ANSWER 19 OF 23 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1989:434215 CAPLUS
DN 111:34215
TI The characterization, partial purification, and regulation of an
NGF - ***activated*** ***protein*** ***kinase*** in PC12
cells
AU Gagne, Elizabeth Rowland
CS New York Univ., New York, NY, USA
SO (1988) 166 pp. Avail.: Univ. Microfilms Int., Order No. DA8825019
From: Diss. Abstr. Int. B 1989, 49(9), 3551-2
DT Dissertation
LA English

L4 ANSWER 20 OF 23 DISSABS COPYRIGHT (C) 2004 ProQuest Information and
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AN 88:18581 DISSABS Order Number: AAR8825019
TI THE CHARACTERIZATION, PARTIAL PURIFICATION AND REGULATION OF AN
NGF - ***ACTIVATED*** ***PROTEIN*** ***KINASE*** IN PC12
CELLS

AU GAGNE, ELIZABETH ROWLAND [PH.D.]; GREENE, LLOYD A. [advisor]
 CS NEW YORK UNIVERSITY (0146)
 SO Dissertation Abstracts International, (1988) Vol. 49, No. 9B, p. 3551.
 Order No.: AAR8825019. 166 pages.
 DT Dissertation
 FS DAI
 LA English
 ED Entered STN: 19921118
 Last Updated on STN: 19921118

L4 ANSWER 21 OF 23 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on
 STN DUPLICATE 13
 AN 1987:340022 BIOSIS
 DN PREV198784048965; BA84:48965
 TI CELL-FREE DETECTION AND CHARACTERIZATION OF A NOVEL ***NERVE***
 GROWTH ***FACTOR*** - ***ACTIVATED*** ***PROTEIN***
 KINASE IN PC12 CELLS.
 AU ROWLAND E A [Reprint author]; MUELLER T H; GOLDSTEIN M; GREENE L A
 CS DEP PHARMACOL, NEW YORK UNIV SCH MED, NEW YORK, NY 10016, USA
 SO Journal of Biological Chemistry, (1987) Vol. 262, No. 16, pp. 7504-7513.
 CODEN: JBCHA3. ISSN: 0021-9258.
 DT Article
 FS BA
 LA ENGLISH
 ED Entered STN: 8 Aug 1987
 Last Updated on STN: 8 Aug 1987

L4 ANSWER 22 OF 23 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on
 STN
 AN 1985:191693 BIOSIS
 DN PREV198529081689; BR29:81689
 TI CELL-FREE CHARACTERIZATION OF A ***NERVE*** ***GROWTH***
 FACTOR - ***ACTIVATED*** ***PROTEIN*** ***KINASE*** .
 AU MULLER T H [Reprint author]; ROWLAND E A; GOLDSTEIN M; GREENE L A
 CS DEP PHARMACOLOGY, NEW YORK UNIV SCH MED, 550 FIRST AVE, NEW YORK, NY
 10016, USA
 SO Biological Chemistry Hoppe-Seyler, (1985) Vol. 366, No. 4, pp. 323.
 Meeting Info.: SYMPOSIUM ON SELECTED TOPICS OF NEUROBIOCHEMISTRY HELD AT
 THE 36TH MOSBACHER MEETING OF THE GESELLSCHAFT FUER BIOLOGISCHE CHEMIE
 (SOCIETY FOR BIOCHEMISTRY), APR. 18-20, 1985. BIOL CHEM HOPPE-SEYLER.
 CODEN: BCHSEI. ISSN: 0177-3593.
 DT Conference; (Meeting)
 FS BR
 LA ENGLISH

L4 ANSWER 23 OF 23 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
 on STN
 AN 85:231664 SCISEARCH
 GA The Genuine Article (R) Number: AFR23
 TI CELL-FREE CHARACTERIZATION OF A NERVE GROWTH-FACTOR (***NGF***)-
 ACTIVATED ***PROTEIN*** - ***KINASE***
 AU MULLER T H (Reprint); ROWLAND E A; GOLDSTEIN M; GREENE L A
 CS NYU, DEPT PHARMACOL, NEW YORK, NY, 10016
 CYA USA
 SO BIOLOGICAL CHEMISTRY HOPPE-SEYLER, (1985) Vol. 366, No. 4, pp. 323.
 DT Conference; Journal
 FS LIFE
 LA ENGLISH
 REC Reference Count: 5
 STN INTERNATIONAL LOGOFF AT 14:24:56 ON 05 NOV 2004